High Resolution Soft X-Ray Microscopy of Micronodules produced by Biomineralization

J. Rothe[1], W. Meyer-Ilse[3], T. Grundl[2], K. H. Nealson[2] and B. P. Tonner[1]
[1]Dept. of Physics, University of Wisconsin-Milwaukee, PO Box 413, Milwaukee, WI 53201, USA
[2]Center for Great Lake Studies, University of Wisconsin-Milwaukee, PO Box 413, Milwaukee, WI 53201, USA
[3]Center for X-ray Optics, Ernest Orlando Lawrence Berkeley National Laboratory,
University of California, Berkeley, CA 94720, USA

INTRODUCTION

The study of the production of inorganic Mn compounds by one-cell micro-organisms is of great importance for the further understanding of the bacterial metabolism based on the respiration of transition metal oxides. The attachment of bacterial cultures to and subsequent modification of inorganic surfaces, as well as the precipitation of inorganic microparticles by these bacteria is a fundamental process in environmental chemistry. Bacterial strains have been discovered which use Mn or Fe compounds as part of their energy transport cycle by oxidizing transition metal compounds to higher valence states, while others will reduce the metal ions [1,2]. An inherent characteristic of the metabolic activity of bacteria is the fact that the chemical processes involved are spatially inhomogeneous. Soft x-ray microscopy is an ideal tool for the investigation of bacterial reaction products. It offers the spatial resolution required, for instance, for the imaging of micro-scale precipitates ('micronodules'). Additionally, by means of the variation of the wavelength (energy) of the incident radiation, the absorption contrast may be adjusted to reveal the spatial distribution of a particular element in the sample. Finally, liquid state imaging of the microorganisms within the so-called 'water window' is possible, giving direct insight into the location of the bacterial activity.

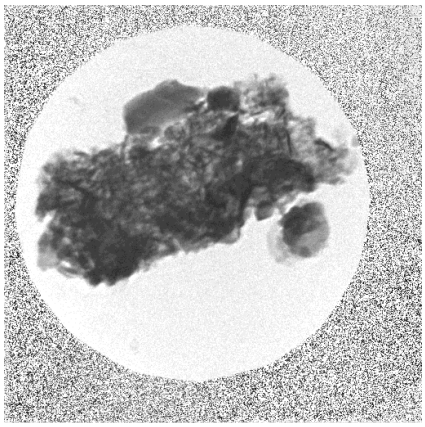

Fig. 1: XM-1 image of micronodules (normalized)

This article is a report on the results obtained for natural Mn nodules (from sediment core samples of Green Bay) produced by the bacterium *S. putrefaciens* using high resolution soft x-ray imaging at the x-ray microscope XM-1, operated by the CXRO at ALS beamline 6.1. The differential energy contrast at the Mn L_{III} absorption edge has been found sufficient to clearly reveal the Mn distribution in a variety of agglomerates of micronodules.

EXPERIMENT

Soft x-ray microscopic images of Mn containing micronodules have been taken at the x-ray micro-scope XM-1, both within the water window (2.4 nm ≅ 516.5 eV) and in the vicinity of the Mn L_{III} absorption edge. As expected from microspectroscopic x-ray absorption measurements performed on these samples [3], the +2 valence state of Mn is predominant. To establish the exact position of the corresponding absorption maximum ('white line') at the given calibration of the XM-1 pinhole monochromator, a sequence of images of a $Mn(II)SO_4$ crystal has been taken between 620 and 646 eV (1.5 eV steps). The ratio of the averaged intensities of two arrays of image pixels (one on top and one next to the edge of the imaged crystal) allows the direct calculation of the absorption coefficient as function of the photon energy.

Fig. 2 shows the resulting x-ray absorption spectrum in the vicinity of the Mn L_{III} edge ($2p_{3/2} \rightarrow 3d_{5/2}$ core transitions). Correspondingly, microscopic images of Mn containing micronodules have been taken at 629.3 eV (1.97 nm), 635.7 eV (1.95 nm), 639.0 eV (1.94 nm - strongest absorption contrast expected) and 642.3 eV (1.93 nm).

One of the major advantages of high resolution soft x-ray microscopy is the possibility to image biological samples in their naturally aqueous environments. For imaging within the water window 2 µl of the centrifuged suspension containing the micronodules have been sandwiched between two Si_3N_4 membranes of 120 nm thickness. All samples have been checked by DIC optical microscopy prior to x-ray imaging.

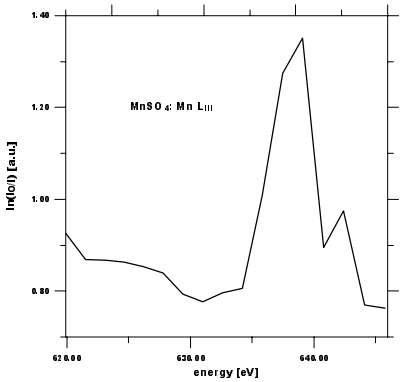

Fig. 2: BL6.1-XM-1 xray absorption spectrum of crystalline $MnSO_4$; absorption maximum at 639 eV.

RESULTS

A typical image of a micronodule of about 10 µm diameter is shown in Fig. 1 (dry sample, 1.97 nm, magnification 2400×). The particles generally tend to form agglomerates of various sizes and shapes, nevertheless the network of needle-shaped structures turned out to be a common characteristic. The image has been normalized by division through a background image, taken at the same wavelength at an 'empty' area of the Si_3N_4 membrane. In Fig. 3 a sequence of images taken at four different wavelengths in the vicinity of the Mn L_{III} absorption edge is presented. By relating to the low energy image (calculation of I_0/I), the Mn distribution appears as bright area in these images. As expected from the sulfate reference measurement, there is no contrast at 1.95 nm (below the white line), maximal contrast at 1.94 nm (on top of the white line, image 3c) and diminishing contrast at 1.93 nm. These images can be seen as a clear visualization of the inhomogeneous action of the Mn reducing bacteria. In all agglomerates, which have been investigated by this technique, the Mn distribution appears to be concentrated in diffuse zones sur-

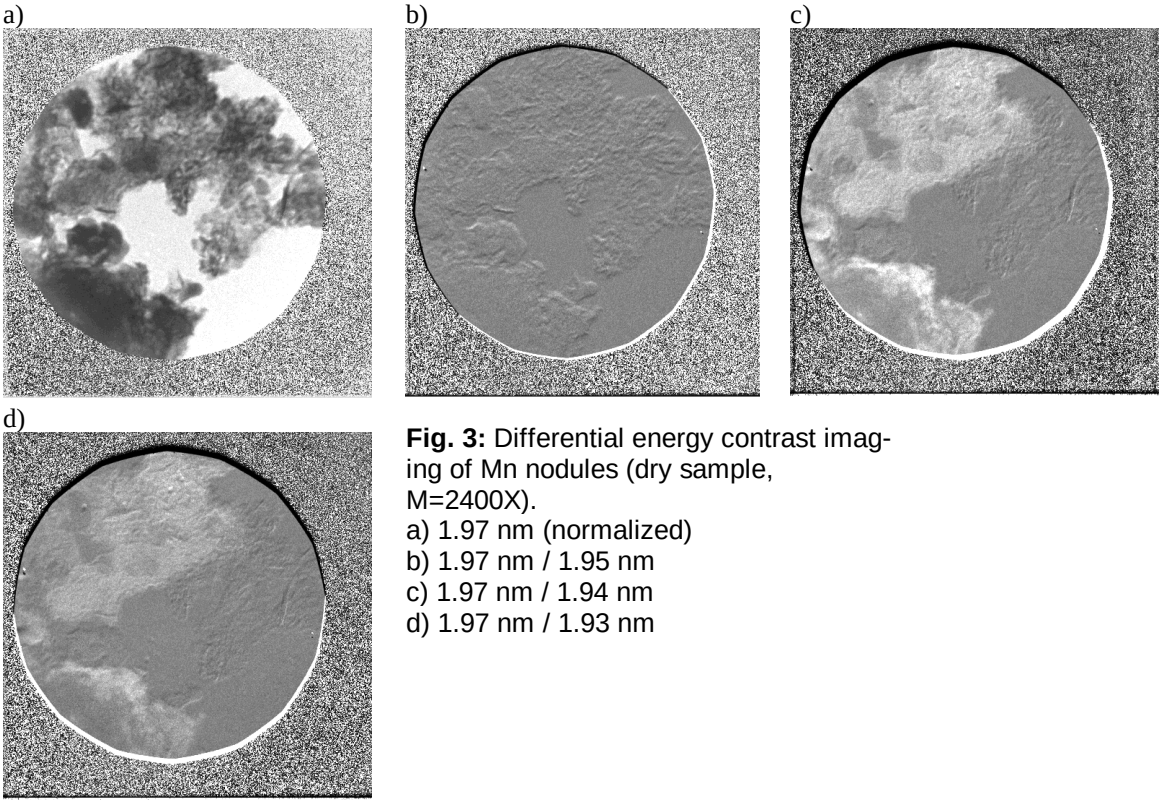

Fig. 3: Differential energy contrast imaging of Mn nodules (dry sample, M=2400X).
a) 1.97 nm (normalized)
b) 1.97 nm / 1.95 nm
c) 1.97 nm / 1.94 nm
d) 1.97 nm / 1.93 nm

rounding larger, regular shaped particles. Here the function of silicate particles as ‘anchors’ for the bacterial attachment and subsequent precipitation of Mn oxides may be observed.

Finally, Fig. 4 shows a wet-cell image of a micro- nodule taken at 2.4 nm inside the water window. The exposure time has been 6.4 sec. The arrow marks a bacterium of the size of about 1 μm still attached to the particle.

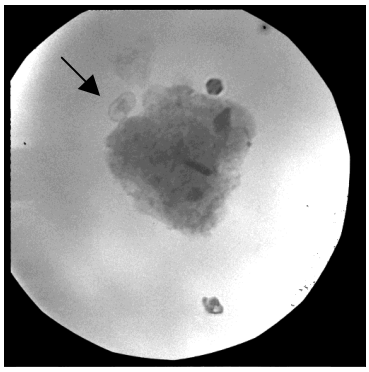

Fig. 4: XM-1 image of micro-nodule taken in the water window (2.4 nm)-the hexagonal structure is an impurity on one of the membranes.

CONLUSIONS

Differential energy contrast imaging at the Mn L_{III} absorption edge has been used to reveal the distribution of Mn in inorganic precipitates produced by one-cell micro-organisms. It has been shown that the investigation of reference compounds helps to establish the energy position of the maximum absorption contrast. The energy resolution of XM-1 turned out to be sufficient to differentiate, in principle, between +2 and +3 Fe species. This may enable valence state selective imaging of transition metal compounds in future work.

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Principal investigator: Brian Tonner, Department of Physics, Laboratory for Surface Studies, University of Wisconsin-Milwaukee. Email: tonner@csd.uwm.edu.